THE EFFECTS OF X-IRRADIATION ON THE FERTILIZED EGGS OF THE ANNELID, CHAETOPTERUS[1]

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The study of ionizing radiations and their effects on living systems has been of interest to biologists for many years. An important manifestation of such effects is evident in the mitosis of cells or tissues exposed to radiation, and excellent experimental material for the analysis of cytological and developmental consequences of such exposure is available in the eggs of a number of marine invertebrate animals. One of the more favorable forms is the polychaete annelid, *Chaetopterus pergamentaceus*. The eggs of this form, although not transparent, are characterized by moderate amounts of yolk, so that it has been possible to make whole-mount preparations of the irradiated and control eggs which show pertinent cytological detail clearly. Furthermore, the normal development of Chaetopterus has been studied by Mead (1898) and Lillie (1906), and there are available time-tables of development for given temperatures, so that irradiation can be begun at a known stage of mitosis, and subsequent deviations from the normal schedule of events studied in considerable detail.

Materials and Methods

Ripe Chaetopterus males and females were collected by the M. B. L. Supply Department, and maintained in the laboratory in separate large fingerbowls supplied with running sea water. Eggs were obtained by clipping off two or three posterior parapodia from a single female; these parapodia were transferred to pieces of cheesecloth wet with filtered sea water, through which the eggs passed into 100 cc. of filtered sea water contained in each of two (control and experimental) plastic boxes, $3\frac{1}{4}'' \times 2\frac{3}{4}'' \times 1\frac{1}{4}''$ in size and provided with lids. The eggs were allowed to stand undisturbed for 15 minutes; during that period, the first maturation division proceeds to the metaphase (Lillie, 1902). Sperm were obtained by placing one parapodium from a male in 100 cc. of filtered sea water, 15 minutes before they were to be used for insemination.

Immediately after insemination, the lids were placed on both boxes of egg suspension, and exactly thirty minutes after insemination, the experimental eggs were irradiated by the Laboratory x-ray technician, Mr. Alan P. Brockway. Samples from both control and experimental groups were examined at intervals with a binocular dissecting microscope, to ascertain the progress of cleavage and later development. After irradiation, the contents of control and experimental boxes were transferred to two small fingerbowls, each containing an additional 100 cc. of

[1] Work done under A. E. C. Contract AT-(40-1)-1085, and under a grant from the National Institutes of Health, RG-3907.

filtered sea water; both fingerbowls were then kept on the sea water table, with running sea water flowing around them. The room air temperature varied from 21 to 25° C. Control and experimental cultures were examined for the final time 19 to 22 hours after insemination, when the controls were vigorously swimming trochophores.

The x-ray generator used operates simultaneously two Coolidge tubes at 25 ma and 182 KVP, with an inherent filtration of 0.2 mm. copper. The plastic box (from which the lid was first removed) containing the experimental eggs was thus "cross-fired" between two x-ray beams. Since the eggs were distributed in an even layer on the bottom of the box, a fairly uniform field of irradiation was possible. The x-rays were delivered at two different rates. For total dosages below 3570 r, the machine was calibrated at 510 r per minute with the two tubes 48 cm. apart, the bottom of the Lucite box being approximately equidistant between the two tubes. For dosages above 3570 r, the x-rays were delivered at a rate of 2160 r per minute, with the tubes 16 cm. from one another. There was no appreciable rise in temperature during any of the irradiation treatments, and artificial cooling measures were therefore not used. The duration of treatment varied from one-half minute to eight minutes, and the dosages used were 255 r to 17,280 r

Cytological preparations were made of samples from the control and experimental cultures, at times calculated to result in fixation of the eggs at the metaphase or anaphase of the first three cleavages. A modified squash whole-mount technique was devised, which yielded excellent preparations in a minimum of time. The method involved fixation of the eggs in Kahle's fluid [2] (water, 30 parts; 95% alcohol, 16 parts; formalin, 8 parts; glacial acetic acid, 1 part) on cover-slips. A single drop of concentrated egg suspension was placed on one clean No. 1 square cover-slip, and a drop of fixative on a second cover-slip; the second cover-slip (with the fixative) was inverted and dropped face-to-face onto the first cover-slip. No pressure was applied, and if drops of the correct size were used there was no distortion of the eggs. After about five minutes, the two cover-slips (still face-to-face) were transferred as a unit to a small staining jar containing fresh fixative, where they were kept for 15 to 30 minutes. During this period, the cover-slips were carefully separated from one another with watchmaker's forceps; approximately one-half the eggs adhered to each of the two cover-slips, and very few were lost during the process of separation. After separation, the cover-slips were placed in porcelain racks, Chen Type A, provided with wire handles, and subsequent steps were carried out by transferring the racks to tall Stender dishes containing the necessary reagents. Hydration of the eggs through 70% alcohol, 50% alcohol and distilled water was followed by staining for four to six minutes in a solution of Harris' acid haematoxylin, diluted 1:4 or 1:5 with distilled water and filtered before each use. No counterstain was used. After staining, the eggs were "blued" in several changes of tap water alkalinized with 1% sodium bicarbonate solution, and dehydrated in three changes of triethyl phosphate,[2] in carbol-xylol and in two changes of xylol; they were mounted in damar. The preparations were studied at magnifications of 150 ×, 300 × and 660 ×, using a Spencer compound binocular microscope with compensating oculars. Approximately 440 slides were prepared and studied.

[3] We are indebted to Dr. Anna R. Whiting for suggesting the use of these reagents.

Results

The results are summarized in Table I.

It is apparent that relatively low doses of x-rays (255 to 1020 r) had no immediately obvious effects on fertilized Chaetopterus eggs. There was a very slight retardation (3–5 minutes) of the first three cleavages in the experimental eggs after 765 r, as compared with the control eggs, but no other visible gross or cytological effects. However, when the control and experimental cultures were examined 19 to 22 hours after insemination, the trochophores were very abnormal in cultures which had received 500 and 765 r, respectively. They were, for the most part, very disorganized masses, with marked ciliary defects; they moved feebly, if at all. Almost all the larvae were dead in the groups which had received 1020 r, and disintegration of the undifferentiated cytoplasm had occurred.

In most of the experiments where the eggs were treated with 1275 r and higher dosages, there was at least a slight retardation (5–10 minutes) of the first three cleavages, and the majority of the embryos were dead 19 hours after insemination. The few surviving trochophores were very abnormal, with ciliary defects, cytoplasmic blebs, etc.

The occurrence of cleavage retardation reported here is in accordance with the results obtained by other workers. Thus, Packard (1918) observed delays in the division of Chaetopterus eggs irradiated with gamma rays from radium. Cook (1939) demonstrated a two- to five-hour delay in the first cleavage of Ascaris eggs x-irradiated at the one-cell stage. Henshaw (1940a, 1940b) and Henshaw and Cohen (1940) described marked cleavage retardation in Arbacia eggs, after x-irradiation of eggs, or sperm, or both gametes. Carlson (1938) described a cessation of mitosis in grasshopper neuroblasts treated with varying doses of x-rays (100–1000 r). The recovery time for return of mitosis (anaphases being used as the criterion) varied from three hours after 100 r to 22 hours after 1000 r. There must be a considerable difference in the radio-sensitivity of Chaetopterus eggs, as opposed to grasshopper neuroblasts, since complete inhibition or even pronounced delay of mitosis in our experiments required much higher dosages.

X-ray dosages below 8640 r did not result in any obvious cytological damage to eggs fixed at the times of the first three cleavages; at and above that dose level, however, there were often multipolar spindles, chromosome bridges at anaphase, and fragmentation, particularly in eggs fixed at the time of the third cleavage. These were very similar to the aberrations described by Costello, Henley and Kent (1952) in P^{32}-treated fertilized Chaetopterus eggs. The chromosomes in the Chaetopterus egg are small, and the detection of minute cytological abnormalities is not always possible.

Eggs treated with 15,120 r presented a striking cytological picture when they were fixed at the time of the second and third cleavages. At least some degree of karyokinesis had apparently taken place, but it was not accompanied by cytokinesis, so that there were often several interphase nuclei present in one undivided or incompletely divided mass of cytoplasm. The nuclei were elongated and somewhat pear-shaped, with the narrow "stem" of one of a pair of nuclei directed toward the narrow "stem" of what was apparently its sister nucleus. One had the impression that an abnormal mitotic division with, apparently, a thick chromatin bridge, had been arrested during its course. This phenomenon of nuclear division without

TABLE I—*X-irradiation of fertilized Chaetopterus pergamentaceus eggs*

X-ray dose*	Gross abnormalities noted at stage of:				Cytological abnormalities noted in eggs fixed at:		
	1st cleavage	2nd cleavage	3rd cleavage	Trochophores	1st cleavage	2nd cleavage	3rd cleavage
255 r	None	None	None	Very slight: a few dead	None	None	None
510 r	None	None	None	Abnormal: ciliary defects, cytoplasmic blebs	None	None	None
765 r	Slight retardation**	Slight retardation	Slight retardation	Abnormal: ciliary defects, cytoplasmic blebs	None	None	None
1020 r	None	None	None	Mostly dead; those living very abnormal	None	None	None
1275 r	Retardation	Retardation	Retardation	Mostly dead; those living very abnormal	None	None	None
1530 r	Slight retardation	Slight retardation	Slight retardation	Dead	None	None	None
2550 r	Slight retardation	Slight retardation	Slight retardation	Mostly dead; those living very abnormal	None	None	None
3570 r	None	Slight retardation	Slight retardation	Dead	None	None	None
8640 r	Retardation	Retardation	Abnormal 2- and 3-cell stages	Dead	None	None	Multipolar spindles; chromosome fragments and bridges
12,960 r	Retardation	Retardation	Retardation	Dead	None	None	Multipolar spindles; chromosome fragments and bridges
15,120 r	Retardation	Retardation	Retardation	Mostly dead; those living very abnormal	None	Nuclear division without division of cytoplasm. Many very elongated interphase nuclei	Multiple interphase nuclei in undivided or partially divided cytoplasm. Many elongated nuclei
17,280 r	Retardation	Inhibition (most eggs not cleaved)	Inhibition (most eggs 2- and 3-cells)	Dead	None	Undivided eggs	Two interphase nuclei present in each of the 2 cells in most cases. Some 3-cell stages with 1 interphase nucleus per cell; AB cell is one which appears to have divided

* X-ray data: Inherent filtration 0.2 mm. Cu; 25 ma; 182 KVP. Two tubes: 48 cm. apart, 510 r per minute, for doses up to 3570 r; 16 cm. apart, 2160 r per minute, for doses above 3570 r; irradiated eggs equidistant between tubes in all cases.

** Cleavage retardation times were ascertained only approximately; in all cases they varied from 4 to 15 minutes, except in those groups in which cleavage was completely inhibited.

cytoplasmic division, or with incomplete cytoplasmic division, is perhaps the most striking effect noted after treatment of these eggs with high dosages of x-rays.

X-irradiation with 17,280 r resulted in marked effects (both gross and cytological) on the first three cleavages. The division to two cells was very abnormal and noticeably retarded (5–7 minutes), and the two subsequent divisions were almost completely inhibited. There were some two- and three-cell stages present in the samples fixed at the times of second and third cleavage in the controls; the stages fixed 75 minutes after insemination often had two interphase nuclei in each of two cells, again indicating a suppression of cytokinesis at an earlier stage. The three-cell stages (which are not normally found in the cleavage of Chaetopterus, although the polar lobe may simulate a third cell) usually had only one interphase nucleus in each of the three cells. It appeared that cytoplasmic division had taken place only in the smaller AB blastomere at the second cleavage, the larger CD blastomere remaining undivided. The possible significance of this observation will be discussed below.

Discussion

Sensitivity of prophase chromosomes to irradiation

The design of our experiments was such that treatment was begun at prophase of the first cleavage, a time which appears, according to the findings of many observers utilizing a variety of materials, to be very susceptible to radiation. This fact, coupled with the circumstance that division in Chaetopterus eggs is predictable and synchronous (within certain limits imposed by temperature and other environmental factors), makes the material favorable for studies of radiation effects.

One of the early reports on the susceptibility of prophase chromatin to irradiation came from Strangeways and Hopwood (1926), who irradiated chick tissue cultures with x-rays and found the most sensitive period to be at the time immediately before the onset of visible prophase. Sax (1938, 1943) reported the greatest frequency of x-ray-induced chromosome aberrations in Tradescantia microspores which were at the meiotic or mitotic prophase at the time of treatment. Luther (1938) x-irradiated frog eggs, and concluded that the maximum susceptibility was during prophase, the minimum susceptibility at metaphase. The findings of Marshak (1939) are somewhat at variance with the general observation that prophase is the stage of greatest sensitivity; x-irradiated onion seedlings, pretreated with dilute solutions of ammonia, were examined cytologically and the results interpreted to indicate that the chromosomes were most sensitive at the resting stage. This was held to be the case regardless of whether or not ammonia pre-treatment was used.

Henshaw and Cohen (1940) x-irradiated fertilized Arbacia eggs, and found that when cleavage retardation was the criterion, the time of greatest susceptibility was during the period after the male and female pronuclei had come together, and during early prophase. The late prophase stage, immediately before breakdown of the nuclear membrane, was found by Carlson (1941) to be the most sensitive period for grasshopper neuroblast cells, and Swanson (1942) made similar observations on Tradescantia pollen tube chromosomes.

More recently, Bloom, Zirkle and Uretz (1955) utilized microbeams of protons and ultraviolet light for irradiation of individual chromosomes and parts of chromo-

somes, in cultures of Triturus heart tissue. They found that a given dose at the prophase stage produced "sticky" chromosomes and chromosome fragmentation; the same radiation to metaphase chromosomes resulted, again, in sticky chromosomes but very few akinetic chromosomes or fragments were observed. Their findings are of great interest, not only because of the highly localized character of the radiation used, but also because they were able to follow the fate of a single irradiated chromosome in considerable and exact detail by the use of phase optics and time-lapse motion pictures.

When Chaetopterus eggs are x-irradiated beginning 30 minutes after insemination, both polar bodies have usually been given off (except in a few of the experiments performed early in the breeding season, when low temperatures slowed down all features of development of the egg). At 21° C., according to Heilbrunn and Wilson (1948), the male and female pronuclei have approached one another and are fusing, and by 40 minutes after insemination, the fusion nucleus is at the prophase of the first cleavage. This time-table of events proceeds at a considerably faster rate when the temperature is increased, and our data indicate that for most of the experiments reported here, the eggs were at the early prophase stage when irradiation was begun.

Possible effects of irradiation on early development of the eggs

In the ovary of the Chaetopterus female, a definite polarization of the egg is evident, the future animal pole being free in the lumen of the ovarian tubule, and the future vegetal pole being attached to the wall of the tubule (Lillie, 1906). Thus, the subsequent position of the polar bodies is "set" very early in development. The cleavage spindle is formed by the separation of the two sperm centrosomes (Mead, 1898), in a position in the egg which is determined by the position of the male pronucleus. This, in turn, is determined by the polarity of the egg (the sperm nearly always entering in the vegetal hemisphere), so that there is an orderly chain of events, each one of which is predicated on the original polarity of the egg.

Lillie (1906) described the separation of large basophilic granules from the chromosomes of the first cleavage in Chaetopterus. The subsequent division of the egg segregates all these granules into the CD blastomere, none being left in the AB cell. X-irradiation with 17,280 r at prophase resulted, as noted above, in a failure of the CD blastomere to divide at the second or at the third cleavage, so that there appears to have been a drastic effect on the distribution of these granules, as well as on other cytoplasmic movements.

Indeed, irradiation of eggs at the prophase of first cleavage could very well have other far-reaching effects, quite apart from those on the chromosomes. The influence of x-irradiation on viscosity changes during mitosis of the Arbacia egg has been described by Wilson (1950). In unirradiated eggs, there is a marked increase in viscosity which reaches a value three or four times that of the unfertilized eggs at 15 minutes after insemination; this increased viscosity persists for a few minutes, and then drops to almost the level of viscosity of unfertilized eggs shortly before the first cleavage. Approximately the same magnitude of increase was observed in eggs irradiated and then inseminated, but it remained high for a period two to three times that of the control eggs, eventually decreasing shortly before

cleavage. If viscosity changes occur in the fertilized Chaetopterus egg irradiated after insemination, they would be expected to have profound effects on the complex patterns of oöplasmic segregation characteristic of this form. Lillie (1906) demonstrated that there was an exact correlation between the distribution of ectoplasmic spherules (which, before the breakdown of the germinal vesicle, are distributed over the animal two-thirds of the egg, and which come to overflow the vegetal hemisphere after germinal vesicle breakdown) and the distribution of cilia in the trochophore larvae. The cilia apparently do not develop directly from the spherules, however. The fact that the later stages of development in our experimental cultures were almost invariably marked by abnormalities of cilia distribution (if, indeed, the embryos survived to the stage of cilia formation at all) indicates that the irradiation directly or indirectly affected some part of the cilia-producing mechanism.

The phenomenon of polar lobe formation, which is characteristic of the eggs of certain annelids (including Chaetopterus) and molluscs, is a striking indication of the profound changes which occur in the cytoplasm of these eggs within the first few hours after fertilization. Although we observed no visible evidence of malformation of the polar lobe in the irradiated eggs, it is quite possible that there were inconspicuous disturbances in the apportionment of cytoplasmic materials to this structure. It is of interest in this connection to point out that even after 17,280 r, polar lobe formation immediately before the first cleavage was morphologically normal, although delayed. Formation of the second polar lobe after this dosage appears to have been completely suppressed.

In any event, the deleterious effects of x-ray treatment on later development are, *per se,* an indication that irradiation interfered with early processes of differentiation.

The possible role of cytoplasmic effects in radiation damage has been considered in the recent paper by Ord and Danielli (1956), in which they transferred x-irradiated *Amoeba proteus* nuclei to non-irradiated cytoplasm of the same form. The resulting organisms had a somewhat lower percentage of survival than did intact irradiated amoebae. When non-irradiated nuclei were transfered to irradiated cytoplasm, 18–48 hours after irradiation, the animals survived despite the fact that they had received very high doses of x-rays; they were able to form new clones, although the time of first division was somewhat delayed. If the transfers of normal nuclei to irradiated cytoplasm were done sooner than 18 hours after treatment, however, the normal nuclei were apparently lethally damaged by the irradiated cytoplasm in most cases. Ord and Danielli point out that in this study, nuclear damage appears to be a *direct* result of x-ray damage to the nuclei, and an *indirect* result of contact with damaged cytoplasm. They suggest that perhaps the importance of nuclear damage from radiation, as opposed to cytoplasmic damage, may have been somewhat over-emphasized.

Chromosome aberrations

There have been many reports in the literature of chromosome aberrations which occur after irradiation from various sources. An early one was the paper by Packard (1918); he irradiated unfertilized Chaetopterus eggs with gamma rays from a radium source and observed multipolar spindles at the first cleavage, after

the treated eggs had been inseminated. Packard interpreted these multipolar spindles as being due to polyspermy, which he felt was facilitated in some way by the irradiation. Alberti and Politzer (1924a, 1924b) described and figured a variety of cytological abnormalities in the corneal epithelium of larval salamanders which had been x-irradiated; among these anomalies were chromatin bridges at anaphase, telophase and interphase, akinetic fragments and chromosomes, accessory nuclei, multiple akinetic chromosomes, and multipolar spindles. Similar effects were described by Strangeways and Hopwood (1926) in x-irradiated chick tissue cultures, and by Sonnenblick (1940) in the embryos obtained after mating x-irradiated male and female Drosophila adults. Laznitzki (1943) also x-irradiated chick tissue cultures, and described a number of cytological abnormalities resulting from treatment; however, this author emphasizes the fact that no multipolar spindles were observed in irradiated material, which is in contrast to the findings of other investigators. Zirkle and Bloom (1953), utilizing proton bombardment of parts of amphibian heart cells in tissue culture, reported chromosome bridges, inhibition of cytokinesis, and unequal distribution of the daughter chromosomes. Chromosome fragmentation after irradiation has been described by Carlson (1938) for grasshopper neuroblast cells, by Fabergé (1940) for Tradescantia pollen grains, by Bishop (1942) for grasshopper spermatocytes, by Whiting and Murphy (1956) for Habrobracon oöcytes, and by Lesley and Lesley (1956) in plants from treated tomato seeds. We have observed many of the aberrations described above in our material, especially after the higher doses of x-rays.

Henshaw (1940d) studied the multipolar spindles which occurred in fertilized Arbacia eggs after x-irradiation; it is interesting that in contrast to our findings (where multipolar spindles clearly attributable to irradiation were observed no sooner than the third cleavage), he described such effects at the first cleavage. This difference may be due to the fact that Henshaw used considerably higher doses (31,200 r) than were employed in the present study. In the same paper (1940d), he found no evidence of nuclear division without accompanying cytoplasmic division at the first cleavage in his treated eggs. We found karyokinesis without cytokinesis only at the second and third cleavages, which apparently were not studied by Henshaw.

Recently, Bloom, Zirkle and Uretz (1955) irradiated parts of chromosomes of amphibian heart cells in tissue culture, using microbeams of protons or ultraviolet. Irradiation of the kinetochore region resulted in "drifting" of the chromosome until anaphase when it was incorporated as a lobe on the daughter nucleus, or became a small accessory nucleus. Chromosome "stickiness" and fragmentation were also reported. Chromosomes treated with beams of protons or ultraviolet outside the kinetochore region showed no such effects. Bombardment of extra-chromosomal areas of the cells (cytoplasm and the ends of spindles) with relatively large numbers of protons produced no effects at the site of irradiation. Heterochromatic ultraviolet irradiation of the same regions, however, resulted in a disappearance of the spindle and derangement of the characteristic metaphase configuration; a "false anaphase" followed, in which chromosomes, rather than chromatids, moved apart.

It is of interest that many of the chromosome aberrations typical of radiation damage are produced also by a variety of other agents including, for example, low temperature (Callan, 1942; Böök, 1945; Henley, 1950; Henley and Costello, 1949);

high temperature (Briggs, 1947); colchicine (Callan, 1942); and ribonuclease (Kaufmann, MacDonald and Bernstein, 1955).

Androgenesis after irradiation

There has been considerable discussion in the literature as to the possibility of androgenesis occurring in fertilized irradiated eggs, as a consequence of irreparable damage to the egg chromatin, so that development proceeds with only the haploid, paternal complement of chromosomes. Packard (1918) reported that in Chaetopterus eggs which had received heavy doses of gamma radiation before insemination, the female pronucleus remained in a polar position, often attached to the polar body material by a fine cytoplasmic strand in which chromatin threads can be distinguished. Cleavage took place in such eggs in an orderly fashion, however, and the haploid number of chromosomes was present. Whiting (1948, 1955) found haploid androgenetic males in Habrobracon. They developed only from eggs x-rayed in first meiotic metaphase, thereby resembling Packard's results in Chaetopterus.

Henshaw (1940d), on the other hand, reported that both pronuclei participated in the development of irradiated Arbacia eggs, even after heavy doses of x-rays. However, Arbacia eggs are fully mature, with the female pronucleus present, when in the fertilizable condition, whereas Chaetopterus eggs are at the metaphase of the first maturation division.

Whiting (1955) studied Feulgen preparations of Chaetopterus eggs irradiated (during metaphase I) with 60,000 r and fertilized 3½ minutes later with untreated sperm. The majority underwent continued cleavage, and of these 26% appeared to have sperm chromosomes, only, and were therefore androgenetic. Whenever chromatin abnormalities appeared in cleaving cells, there were more than nine chromosomes (the haploid number) present, and in all cells in which there were nine chromosomes, only, no aberrations were visible. Whiting therefore found no evidence of an injurious effect of irradiated cytoplasm upon untreated chromosomes. In the experiments reported in the present paper, apposition of the pronuclei occurred normally in irradiated eggs, and the diploid number of chromosomes appeared to be present. Since the eggs had been inseminated before irradiation, the occurrence of normal fertilization implies that no damage was suffered by the sperm, either directly or as a consequence of its passage through irradiated egg cytoplasm.

The effects of irradiation on later development

Lea (1955) points out that the death of a cell as a result of irradiation usually does not occur immediately but at, or following, the next division of the cell; an immediate lethal effect requires much larger doses of radiation than a delayed lethal effect. The results obtained in the present study confirm this general statement, and are in accordance with the findings of other investigators. Cook (1939), for example, x-irradiated Ascaris eggs at the one-cell stage and obtained highly abnormal embryos, consisting of unorganized masses of cells. Sonnenblick (1940) reported the occurrence of undifferentiated non-viable masses of cells among the progeny of adult fruit flies which had been treated with x-rays. Henshaw (1940c) found that x-irradiation of Arbacia eggs with 14,400–28,800 r resulted in the

appearance of a wide gradation of effects at the pluteus stage, ranging from shortening of the skeletal arms to the formation of a disorganized mass of cytoplasm which subsequently disintegrated. Giese (1946) treated Chaetopterus sperm with 8000–16,000 ergs/mm² of ultraviolet; when such sperm were used to inseminate normal eggs, death ensued, at a stage which is not specified.

Even after x-ray doses as low as 255 r in our experiments, there was some mortality in Chaetopterus trochophores examined approximately 22 hours after insemination. Above that dose level, the larvae were very abnormal or, more commonly, dead. This delayed action was especially striking after some of the lower doses of x-rays, where no particular gross or cytological effects (except for slight cleavage retardation) were discernible at earlier stages after treatment.

Summary

1. Fertilized eggs of Chaetopterus were x-irradiated, beginning 30 minutes after insemination; doses from 255 r to 17,280 r were used, and the duration of treatment was one-half minute to eight minutes. Observations were made of both living and fixed eggs, at various intervals after irradiation, and of living trochophore larvae 19–22 hours after irradiation.

2. The principal effects of relatively low doses (255–1020 r) were found to be a slight retardation of cleavage (3–5 minutes), and the production of abnormal trochophore larvae which were characterized by severe ciliary defects, cytoplasmic blebs, and very feeble movements (following doses of 255 to 765 r). Doses of 1020 r and above resulted in death of most of the larvae by the trochophore stage. The majority of the eggs irradiated with 1275 r and above showed at least a slight retardation of the first three cleavages.

3. Among the cytological abnormalities observed (especially after the higher doses) were multipolar spindles, chromosome fragmentation, and karyokinesis without cytokinesis.

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